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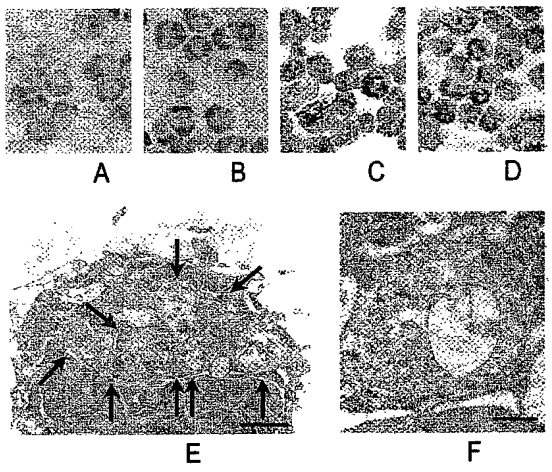
(54) Title: MAGNETIC RESONANCE TRACKING OF MAGNETICALLY LABELED CELLS



(57) Abstract: The method of the present invention is to label living cells so as to render them detectable in MRI. The invention relates to the use of magnetic resonance (MR) sensitive agents to render treated living cells identifiable and distinguishable in MR Imaging (MRI). MR sensitive agents can be bound to a targeting compound such as a protein or antibody. The targeting compound reacts with living cells in a way that the MR sensitive agent is incorporated into the or onto the cell surface. The MR sensitive agents can also be coated with agents that render the magnetic resonance sensitive agent able to bind to or be internalized by cells. The treated cells of the invention can be applied to patient for any therapeutic, diagnostic, or experimental purpose. The invention also encompasses imaging the host with MRI to locate MR labeled cells, to track their migration or bio-distribution non-invasively, and to establish to survival characteristics of such cells.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

MAGNETIC RESONANCE TRACKING OF MAGNETICALLY LABELED CELLS

RELATED APPLICATION

The present application claims the priority of U.S. Provisional application serial number 60/135,428, filed May 21, 1999, which is herein incorporated by reference.

FIELD OF THE INVENTION

The invention relates to the use of magnetic resonance (MR) sensitive agents to render treated living cells identifiable and distinguishable in MR Imaging (MRI). MR sensitive agents can be bound to a targeting compound such as a proteins or antibody. The targeting compound reacts with living cells in a way that the MR sensitive agent is incorporated into the or onto the cell surface. The MR sensitive agents can also be coated with agents that render the magnetic resonance sensitive agent able to bind to or be internalized by cells. The treated cells of the invention can be applied to patient for any therapeutic, diagnostic, or experimental purpose.

TECHNOLOGY REVIEW

Many therapeutic and diagnostic strategies are based upon introducing exogenous living cells or tissues into a patient or host. The object is either to supply cells or tissue that the patient is lacking or to supply cells that make a natural substance that the patient is lacking or needs to have supplemented. Examples of such procedures are transfusion, organ and tissue transplantation, fetal or embryonic cell transfusion and tissue transplantation, and stem cell therapies. Currently used procedures include blood and blood components or white blood cell transfusions; heart, lung, and liver transplantation; and skin and bone grafts. Such strategies may supply the only therapy for some disease states. With the advent of fetal cell and tissue therapies, such as current treatment of Parkinson's Disease, and with the discovery of many kinds of stem cells and progenitor cells, such as neural stem cells and mesenchymal stem cells, the use of such therapies will expand to treat many disease states currently untreated or under-treated.

An important under-treated clinical area is morbidity of the nervous system. This is due to the inability of much of the nervous system to regenerate or repair itself. Demyelination is a common pathological finding in human neurological diseases and frequently persists as a result of failure of endogenous repair. It is an important pathological component of multiple sclerosis (MS) and the inherited leukodystrophies. Recent research suggests that it is possible to promote (re) myelination in animal models of abnormal myelination, either by endogenous oligodendrocytes or exogenous myelinating

cells, (*D.J. Miller et al., Brain Pathol. 6, 331 (1996); I.D. Duncan et al., Mol. Med. Today 3, 554 (1997)*). The latter repair mechanism has received particular attention, since it has been shown that transplanted oligodendrocyte precursor cells can myelinate large areas in the central nervous system (CNS) (*I D. Duncan and E.A. Milward., Brain Pathol. 5, 301 (1995); I.D. Duncan., Neuropathol. Appl. Neurobiol. 22, 87 (1996)*). A similar therapeutic approach in humans could be pursued and is supported by the safety and effectiveness of early human neurotransplantation studies, (*O. Lindvall et al., Science 247, 574 (1990); H. Widner et al., New Engl. J. Med. 26, 1556 (1992); O. Lindvall et al., Ann. Neurol. 35, 172 (1994); J.H. Kordower et al, N. Engl. J. Med. 332, 1118 (1995); C.W. Olanow et al,. Trends Neurosci. 19, 102 (1996); G.K. Wenning et al., Ann. Neurol. 42, 95 (1997); R.A. Hauser et al., Arch. Neurol. 56, 179 (1999); A.C. Bachoud-Levi et al., Progress in Brain Res.. 117, 511 (1998)).*

Similarly, neural stem cells have recently been discovered and may have many uses in the treatment of neural and nervous system injury and disease. Some potentially treatable conditions, which are now under-treated, include stroke, spinal cord injury, peripheral nerve injury, myelin diseases including demyelinating diseases, Parkinson's disease, Huntington's disease and Alzheimer's disease.

A problem common to all therapeutic strategies involving application of exogenous cells is identifying and monitoring the cells in the host. It is currently difficult or impossible to monitor the location of such cells or tissues in the host after application. It may also be difficult to establish the survival of these cells in the host. Some current strategies rely on post mortem analysis and verification of transplanted material. This problem must be overcome before such therapies can achieve their full potential, in particular where human health is involved. Currently available procedures to locate transplanted cells are invasive and destructive.

Another area of current research, which uses exogenously applied cells, is application of cells that have been transfected or otherwise loaded with vectors to make proteins or drugs. This strategy can be used to treat a disease which state is caused by the lack of a certain protein or natural product, such as diabetes. It could also be used when the treatment of a disease required a drug or protein to be continuously applied to a certain place, such as treating cancer or heart disease. Developing such strategies will require the ability of researchers to target and monitor not only the location of the loaded cells but also

their continued viability and thus output. It is currently impossible to monitor the course of these therapies.

SUMMARY OF THE INVENTION

The present invention describes a method for labeling living cells to render them identifiable and distinguishable by MRI. Such cells can then be exogenously applied to a host and monitored within the host using MRI. The cells can be applied for diagnostic, therapeutic, or research purposes. The method involves combining a magnetic resonance (MR) sensitive label to a targeting compound, such as a ligand or antibody, which binds to the surface of the cell. The cell can then internalize the label/targeting compound complex. The treatment with the MR sensitive agent can further comprise mixing coating or reacting the MR sensitive agent with molecules such as polymers, including proteins, so as to allow it to bind with receptors or other structure on the cell surface.

An object of the invention is therefore to enable researchers and clinicians to monitor, as extensively and repeatedly as necessary, the movement, location, and survival of exogenously applied living cells. A method is also described to infuse or otherwise apply the cells into the host and to scan the host to locate the labeled cells. The cells may be incorporated into a tissue or organ, or introduced into a tissue or organ that is then transplanted into a host. The host is scanned with MRI to identify and monitor the movement and disposition of the cells in the body. Repeated scans as necessary to the clinical condition of the host or as required for experimental or safety purposes are within the scope of the invention. A non invasive method for monitoring exogenous neural material would allow monitoring of therapeutic cells to verify their location and would enable procedures not currently possible.

In the (re) myelination studies referred to above, the clinical outcome of such studies will be directly determined by the extent of myelination, and thus by the immediate dispersion, migratory capacity, and long-term survival of grafted cells. The migratory capacity of transplanted cells is of key importance in determining the extent of (re) myelination and can, at present, only be evaluated using invasive and irreversible procedures. A technique that could monitor grafted cell migration continuously and non-invasively will be crucial to guide further advances in neuro-transplantation research.

In another aspect, the invention relates to methods to label cellsin the host, *in situ*, so as to allow labeling of structures in the host. This would allow monitoring of labeled

structures and cells. For example, tumors could be so labeled to monitor effectiveness of treatment of treatment and to follow metastasis.

The invention includes a method of labeling living cells to render them magnetic resonance sensitive which encompasses: treating a magnetic resonance sensitive agent to render it capable of being internalized by the cells; bringing the treated magnetic resonance sensitive agent into contact with the cells to be labeled; and allowing the magnetic resonance agent to be internalized by the cell. This is understood to include a magnetic resonance sensitive agent that is a superparamagnetic agent, such as MION-46L. The method of treatment of the magnetic sensitive agent can be to conjugate it to a targeting compound. Such a compound could be an antibody. Some possible antibodies that can be used for the present invention are: 83-19(anti-insulin receptor), JSB-1, MRK-16, C219 (anti-gpl70), OX-26, B3/25, T56/14, OKT-9, L5.1, 5E-9, R17-217, and T58/30, 8D3, and R17-217 (anti-transferrin receptor). Alternatively, the method of treatment of the magnetic sensitive agent is to incorporate it into a magnetodendrimer.

Some of the cells that can be labeled by the present invention are: stem cells, neural stem cells, bone marrow cells, hematopoietic cells, tumor cells, lymphocytes, leukocytes, granulocytes, hepatocytes, monocytes, macrophages, fibroblasts, mesenchymal and neural cells

An additional step when dealing with antibodies is allowing targeting compound to bind to a receptor on the surface of the cell, wherein the receptor is one of the group of hormone, cytokine or growth factor receptors. The targeting compound can bind to a receptor on the surface of the cell, such a receptor could include: insulin receptor, p-glycoprotein, Na^+ /K^+ ATPase, ferritin receptor, lacto-ferritin receptor, transferrin receptor, and VEGF, BDNF, IGF, IL-2, EGF, NGF, and PDGF receptors.

One use of labeled cells is for detecting pathology in a host suspected of having pathology. This is accomplished by labeling living cells with a magnetic resonance sensitive agent; introducing such cells into the host; and imaging the host with MRI so as to locate the cells within the host. For example, carcinoma cells can be so used in an experimental animal.

The invention further encompasses a diagnostic kit for labeling living cells which are to be introduced into a host for diagnostic or therapeutic purposes, such a kit would include: a magnetic sensitive agent which has been treated to render it able to be internalized by the cells; sterile solutions containing one or more of: buffers, salts, proteins,

nutrients, indicator dyes, and preservatives; and sterile containers to bring the magnetic sensitive agent into contact with the cells. Such a kit could include a magnetic sensitive agent incorporated into magnetic dendrimers or complexed to a targeting agent such as an antibody.

The method of the present invention is used for detecting living cells in a host by labeling them with magnetic sensitive agents. Cells can be labeled *in vitro*, followed by applying the cells to the host. The cells could be therapeutic cells including neural stem cells and neural stem cells. In a preferred embodiment the cells are oligo-spheres. The cells can also be hepatocytes.

In another aspect the living cells could be labeled to render them magnetic resonance sensitive by coating magnetic nanoparticles with strepavidin or avidin; biotinylating the cells; contacting the cells with the coated nanoparticles; allowing the cells to internalize the nano-particles; and imaging the cells with MRI.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A and 1B: In A, trypsinized CG-4 cells were labeled for 48h with 0.5 mg Fe/ml unconjugated MION-46L In B, numerous iron-containing vesicles are visible. E is TEM of MION-46L-OX-26 tagged CG-4 cells. Vesicles are indicated by arrows, they measure approximately 0.6-1.0 μm in diameter, and are filled with the electron-dense magnetic nanoparticles. One of the vesicles (double arrows) is shown at a higher magnification in F to demonstrate the association of particles with a (reversed) endocytosed membrane. Bars represent 1 μm in E and 200 nm in F.

Figure 2A and 2B: Graph A shows 1/T1 and Graph B shows 1/T2 as function of Larmor frequency for MION-46L- (0.5 mg Fe/ml) and MION-46L-OX-26 (0.05 mg Fe/ml) labeled cells.

Figure 3A, 3B, and 3C: Magnetic Resonance Image of *Md* rat 10 days following transplantation of magnetically labeled CG-4 cells. A shows the three MRI planes of view at 78 μm isotropic resolution. The contrast in the transverse images (enlarged in bottom row, shown is each 3rd interleaved slice) corresponds to the area of Prussian Blue B and anti-PLP staining C. The inserts in B and C show cell migration from the injection site towards the dorsal column, where the majority of the newly formed myelin was found. (Bars represent 100 μm in the inserts of B and C).

Figure 4A, 4B, 4C, and 4D: *Md* rat 14 days following transplantation. Shown is the sagittal MRI plane A, consecutive slices) at 78 μm isotropic resolution, with cellular migration over

a distance of 4.5 mm. The anti-PLP stains are shown in B-C. In B, note the injection track with cell migration towards the dorsal column (the spherical appearance of the injection track in the MR images is due its direction relative to the orientation of the external magnetic field gradient). Prussian Blue-positive cells D in the area of new myelination resembled the cellular morphology of oligodendrocytes. Bars represent 1 mm in B and C, and 10 μm in D.

Figure 5A and 5B. Graph A shows 1/T1 and Graph B shows 1/T2 as functions of the dose of MION-96-L-OX-26 for several different Larmor frequencies. The highest dose of unconjugated MION-96-L is included for comparison.

Figure 6 MRI of oligosphere migration on days 17 to 27, comparing normal rats to shaker rats.

Figure 7: *In vivo* MR imaging with magnetically labeled, implanted tumor cells.

Figure 8: High-resolution ex vivo MR imaging of a magnetically labeled tumor specimen shows individual cells as single pixels (black dots) going to the periphery from the center.

DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention is to apply a magnetic resonance (MR) sensitive agent to a living cell to give the cell greater contrast in magnetic resonance imaging (MRI). The invention includes labeling living cells to render them magnetic resonance sensitive. The steps of labeling a cell include first treating a magnetic resonance sensitive agent to render it capable of being internalized by the cells; then bringing the treated magnetic resonance sensitive agent into contact with the cells to be labeled; and finally allowing the magnetic resonance agent to be internalized by the cell. These steps have the effect of rendering cells magnetic resonance sensitive. The MR sensitive agent can be paramagnetic, such as gadolinium chelates, or ferrimagnetic, such as iron oxide. Contrast in magnetic resonance is determined by the relaxation rate, which is measured using a relaxometer. MR sensitive agents have the property of enhancing the relaxation rate of a fluid or structure in which they are present. A higher relaxation rate produces a higher degree of contrast and visibility in a MRI scan. The unit of efficiency is plotted as the relaxation rate so that a higher degree of efficiency means more labeling and greater contrast.

Preferably the iron oxide is a superparamagnetic iron oxide. Superparamagnetic iron oxides are (on a millimolar metal basis) the most MR-sensitive tracers currently

available *(R. Weissleder, et al., Adv. Drug. Del. Rev., 16, 321 (1995); J.W.M. Bulte and R.A. Brooks, in Scientific and Clinical Applications of Magnetic Carriers, eds. Häfeli, U., et al., Plenum Press, New York (1997))*. These particles possess a large ferrimagnetic moment that, because of the small crystal size, is free to align with an applied magnetic field (i.e., there is no hysteresis) - hence the term "superparamagnetic". The aligned magnetization then creates microscopic field gradients that dephase nearby protons and shorten the T2 nuclear magnetic resonance (NMR) relaxation time, over and beyond the usual dipole-dipole relaxation mechanism that affects both T1 and T2 relaxation times. Some supermagnetic iron oxides, which may be used in the current invention, are (magneto) ferritins, (magneto) liposomes, (magneto) dendrimers, dysprosium, gadolinium-or-iron-containing macromolecular chelates. In a preferred embodiment, the superparamagnetic iron oxide is MION-46L. MION-46L is a dextran-coated magnetic nanoparticle with a superparamagnetic maghemite- or magnetite-like inverse spinel core structure, measuring 4.6 ± 1.2 nm in diameter. The overall particle size is 8-20 nm. It has the following characteristics i) it is mono-crystalline and small sized, ii) its magnetic properties and effect on NMR relaxation are relatively well understood *(T. Shen et al., Magn. Reson. Med. 29, 599 (1993)*; *R. Weissleder, US Patent 5,492,814 (1996)*; *A. Roch, thesis, University of Mons-Hainaut (1994)*; *S.H. Koenig and K.E. Kellar., Magn. Reson. Med. 34, 227 (1995)*; *J.W.M. Bulte et al., J. Magn. Magn. Mat., 194, 215-221 (1999)*; *A. Roch et al., J. Chem. Phys. 110, 5403 (1999))*, and iii) it forms covalent bonds with monoclonal antibodies, (R. *Weissleder et al., Radiology 182, 381 (1991); L.G. Remsen et al., Amer. J. Neuroradiology 17, 411 (1996); J.W.M. Bulte et al., Proc. ISMRM Sixth Annual Meeting, 307 (1998)*).

The MR sensitive agent is treated in a way as to allow it to be bound to or internalized by a living cell that is to be labeled. This treatment can be coating, opsonization, or conjugation with a targeting compound. A targeting compound can be any compound that reacts with the cell. Conjugation is understood to include co-valently or non-covalently linking the targeting compound to the MR sensitive agent. Preferably the MR sensitive agent is treated by conjugating it to a targeting compound that binds to the cell surfaces. Suitable targeting compounds include but are not limited to proteins, antibodies, hormones, and ligands. MR sensitive agents can also be treated to form magnetodendrimers by any means known in the art, for example the method of Bulte *et al.*, (*Magneto Dendrimers as a New Class of Cellular Contrast Agents. Pro. Internat. Soc. Mag. Reson. Med., 2062, 8th meeting (2000)*).

The method encompasses targeting and endocytosis of the treated MR sensitive agent by means of a specific receptor on the cell surface. Receptors that can be targeted in this way include but are not limited to: insulin receptors, p-glycoprotein receptors, Na^+ /K^+ ATPase receptors, ferritin receptors, lacto-ferritin receptors, G-protein receptors, and transferrin receptors. Other possible receptors are hormone, cytokine or growth factor receptors, such as VEGF, BDNF, IGF, IL-2, EGF, NGF, PDGF receptors. In a preferred embodiment, the MR sensitive agent is conjugated to a targeting compound that binds to the transferrin receptor (Tfr).

Tfr has the following characteristics: i) it is a high-density receptor on immature oligodendrocytes, (*A. Espinosa de los Monteros and B. Foucaud. Dev. Brain Res. 35, 123 (1987); A.J. Roskams and J.R. Connor, in Myelination and Dysmyelination (1990); A.J. Roskams and J.R. Connor, J. Neurosci. Res. 31, 421 (1992)*); ii) it can be recycled (back to the cell membrane) within minutes following endocytosis, allowing multiple delivery of (magneto) pharmaceuticals through use of a single receptor, *(E. Wagner et al., Adv. Drug Del. Rev. 14, 113 (1994))*; iii) iron-regulatory proteins (IRPs) strictly control its expression; iv) the Tfr number can thus tentatively be easily manipulated in culture by varying the amount of iron; and v) it is a ubiquitous receptor present on nearly every mammalian cell, potentially allowing the Tfr approach to be easily extended to MR mapping of many transplanted cell types.

The targeting compound of the invention can be one that binds to the receptor, such as the natural ligand of the receptor, or portions thereof that bind to the receptor. The targeting compound can also be an enzyme, nutrient, antibody, chimeric antibody or portions thereof that bind to the receptor. The term antibody encompasses both polyclonal (poab) and monoclonal (moab) antibodies. Some antibodies encompassed by the invention are: 83-19(anti-insulin receptor), JSB-1, MRK-16, C219 (anti-bpl70), OX-26, B3/25, T56/14, OKT-9, L5.1, 5E-9, R17-217, and T58/30, 8D3, and R17-217 (anti-transferrin receptor). A preferred targeting compound is a monoclonal antibody. The most preferred antibody is OX-26. OX-26 is a mouse IgG2a moab, produced following immunization with lectin-stimulated rat lymph node cells. It binds non-competitively with transferrin and induces internalization of the receptor.

The living cells of the present invention are those that are of therapeutic, diagnostic, or experimental value when introduced into a patient or host. The term cell is understood to mean embryonic, fetal, pediatric, or adult cells or tissues, including but not limited to, stem

cells, pluripotent stem cells, precursor cells, and progenitor cells. It is also understood that the term cells encompasses virus particles and bacteria. The term host can mean any mammalian patient or experimental subject, including human patients or subjects. The living cells of the current invention can be bone marrow cells, hematopoietic cells, tumor cells, lymphocytes, leukocytes, granulocytes, hepatocytes, monocytes, macrophages, fibroblasts, neural cells, mesenchymal stem cells, neural stem cells, and combinations thereof. In a preferred embodiment the cells are neural cells. It is understood that the term "neural cells" includes neurons and neuroglia. The term neuron encompasses central and peripheral neurons, autonomic neurons, and neuroepithelial cells. The term neuroglia encompasses oligodendrocytes, astrocytes, ependymal cells, microglia, stellate cells, Schwann cells, and neurilemma. Preferably the cells are oligodendrocyte progenitors. Oligodendrocytes are glial cells of the central nervous system, which form sheaths around nerve fibers and capsules around nerve cell bodies.

The cells can be applied to the host to cure or diagnose a disease or to supply cell type that is lacking or deficient in the host. The cells can also provide a drug or substance that is needed in the host for diagnostic, therapeutic, or experimental purposes. Cells can be immune cells to specific proteins in the host's body, such as proteins found in malignant tissue or molecules associated with a disease state such as bacterial or viral proteins or glycoproteins.

In a preferred embodiment the cells can be stem cells. Stem cells are cells that retain their ability to divide and to differentiate into specialized mature cells. Preferably the cells are multipotent cells from the nervous which retain their ability to differentiate into oligodendrocytes. Oligodendrocytes are glial cells that mylinate or provide protective sheaths to neurons and axons in the central nervous system.

In another aspect the cells are carcinoma cells. Such cells are neoplastic and divide indefinitely. Preferred tumor cells is small cell carcinoma cells. Labeling such cells *in vitro* is an experimental tool to study how such cells behave in experimental conditions. Cancer cells can also be labeled *in vivo* to allow clinical investigators to track possible metastases.

In another aspect, the present invention encompasses the method of applying such cells, which are labeled with a MR sensitive label, for any for therapeutic, diagnostic, or experimental purposes. The cells can be dispersed, or can be part of a tissue or organ or labeled cells can be applied to any tissue or organ after the cells are labeled. The cells can be directly applied to the area to be treated or studied by means of surgery or injection into

the circulation or injection into a structure, organ, or body cavity *in situ*. When cells are integrated *ex vivo* into a tissue or organ, such tissue or organ can then be surgically applied or transplanted into a host. Preferably the cells are applied directly to a body structure. Most preferably the cells are applied to the central nervous system.

The cells can also be labeled *in situ* for therapeutic, diagnostic, or experimental purposes. The present invention also encompasses infusing magnetic resonance sensitive agents that have been treated to render them capable of being internalized by living cells. Such agents can be infused into such areas as tumors, so that the growth, metastasis, or regression of the tumor can be monitored. Such a procedure could be part of a treatment protocol to monitor disease progress.

The invention encompasses using MRI to monitor the movement, disposition and survival of the cells in the host. When cells are used that are immune cells, which react with a component of a disease process in the host, MRI monitoring can be used diagnostically to locate the cells attached to the disease process in the host. Immune cells are understood to encompass lymphoid or myeloid hematopoietic cells. Examples of such disease processes are malignant and metastatic diseases, degenerative diseases and infectious diseases. The cells can be used experimentally, in animal hosts, to study the development of disease as a basis for designing therapeutic strategies. Tumor or neoplastic cells can be applied to animals as an experimental techniques to study the behavior of neoplastic growth and metastasis in the organism.

Cells can be used to replace injured or diseased cells in the host, examples are diseases of the nervous system, injuries to the nervous system, injuries or diseases of bone, muscle, heart, circulation, internal organs, skin, interstitial tissue, mucosa, lungs, and gastrointestinal tract. When cells are used in this way, the host can be scanned with MRI to establish the location of the cells the movement or migration if any of the cells, and the survival of the cells. The host can be scanned with MRI as frequently and during as long a period of time as required or desirable to monitor the cells. Cells that are loaded with therapeutic vectors can be monitored as necessary to establish their migration if any in the host and to establish their continued survival and ability to produce therapeutic proteins or drugs in the host.

The method encompasses maintaining the cells in buffers and media with inactive ingredients and other components that the cells in question are known to require to preserve their integrity and viability. This can include cell culture media such as Dulbecco's

Modification of Eagles Medium, etc. It can further include components that allow cells to be frozen or preserved such as ethylene glycol, dimethyl sulfoxide, and ethanol. The method includes incorporating the cells into fluid or media to allow their application to a host. Cells may be labeled and then frozen for use. Such cells can be stored in cell banks for distribution or as single dose aliquots. The method encompasses incorporating the cells into applicators, vials, syringes and the like to allow them to be supplied in a consistent or reproducible manner to clinical centers. The method further encompasses supplying cells in a similar manner for experimental purposes, including "test kits" supplied to researchers and clinicians, containing all components required to use the cells. Similar "kits" could be provided to allow end user's to label cells of their choice. Such kits would provide a magnetic resonance sensitive agent that has been treated to allow it to be internalized by cells, along with solutions including buffers, salts, nutrients, stabilizers, and so forth The kits would also contain such applicators and containers as required to perform labeling.

DEFINITIONS

The following definitions are intended to clarify the examples and not to limit the scope of the invention.

avidin *Biochemistry.* a protein isolated from raw egg white that acts as a vitamin antagonist to biotin, binding tightly to it and thereby rendering it unavailable to the body (producing the syndrome known as biotin deficiency).

avidin-biotin technique *Immunology.* a system designed to detect antigens using the strong binding affinity of avidin and biotin being coupled to specific antibodies.

biotin *Biochemistry.* $C_{10}H_{16}N_2O_3S$, a B vitamin that functions as a coenzyme and is ubiquitous in nature. Also, VITAMIN H, COENZYME R.

conjugate joined together; paired; specific uses include: *Chemistry.* **1.** of an acid and a base, related to each other in that the loss of a proton converts the acid into the base, and the gain of a proton converts the base into an acid.. *Immunology.* a substance formed by the covalent bonding of two or more types of molecules, such as fluorescein coupled with an antiglobulin molecule.

embryoid bodies spheres or clumps of embryonic cells.

maghemite *Mineralogy.* Fe_2O_3, a highly magnetic, brown, cubic mineral, massive in habit, having a specific gravity of 4.90 and a hardness of 5 on the Mohs scale; dimorphous with hematite; found as a secondary mineral in Gossans.

myelin *Neurology.* the substance of the cell membrane of Schwann's cells that coils to form the myelin sheath. Also, WHITE SUBSTANCE OF SCHWANN. *Histology.* any of various lipid substances found in normal or pathologic tissue, differing from fats in being doubly refractive.

metastasis *Pathology.* the spreading of a disease from one organ or part to another that is not directly connected with the first organ or part; this may be due to the transfer of microorganisms or to the transfer of cells. *Oncology.* **1.** specifically, the movement of tumor growth from an original primary location in the body to a secondary location via the lymph system or blood circulation. specifically, the movement of tumor growth from an original primary location in the body to a secondary location via the lymph system or blood circulation. **2.** any growth or tumor that develops in this way any growth or tumor that develops in this way.

neoplasm *Biology.* any new and abnormal growth. *Oncology.* specifically, growth that shows cellular proliferation, that grows at a more rapid rate than normal, that continues to grow after the instigating factor is no longer present, that shows a lack of structural organization and coordination with the normal tissue, and that usually creates a mass of tissue, which may be either benign or malignant.

receptor *Physiology.* a specialized sensory nerve structure (exteroceptor, interoceptor, or proprioceptor) at the peripheral end of a sensory neuron that responds to specific types of stimuli. *Cell Biology.* a site in a cell, usually on a membrane that combines with a chemical to specifically alter cell function.

oligodendroglia *Neurology.* small non-neural supporting cells of the nervous system, having spheroid nuclei and delicate cytoplasmic branches, that envelop axons to form myelin sheaths. Also, OLIGOGLIA.

stem cell *Developmental Biology.* a cell, capable of both indefinite proliferation and differentiation into specialized cells, that serves as a continuous source of new cells for such tissues as blood and testes.

streptavidin *Biochemistry.* a protein that is derived from *Streptomyces avidinii* and can be used in place of avidin.

EXAMPLES

EXAMPLE 1

Labeling Oligodenrocytes with OX-26 conjugated MION-46L

OX-26, a targeting compound, was covalently linked to MION-46L a MR sensitive label, using the periodate-oxidation and borohydride reduction method; the alcohol groups of the dextran-coated MION-46-L were covalently linked to the terminal amine groups of OX-26. MION-46-L in 0.01 M citrate buffer, pH = 8.4 was first oxidized at 4 °C for 24 hr with 1 mg $NaIO_4$ per mg Fe. MION-46-L was then purified using a Sephadex G-100 column, removing any unbound or released free dextran. OX-26 in 0.2 M sodium bicarbonate buffer, pH = 6.5, was allowed to form Schiff's bases with MION-46L at 4 °C for 16 h at a 1:1 protein to Fe weight ratio. The unstable Schiff's bases were reduced to secondary amide bonds by adding 1 mg $NaCNBH_3$ per mg Fe and incubation at room temperature for 4 h, and the resulting MION-46L-OX-26 was purified over a Sephadex G-100 column.

Oligodendrocyte progenitors have a greater migratory and myelinating capacity than neonatal cells. The rat oligodendrocyte progenitor cell line CG-4 was chosen as a graft, with the *md* rat as a recipient, since the migration pattern of $LacZ^+$ CG-4 cells has been documented in detail using this model. The *md* rat carries an X-linked recessive mutation at the proteolipid protein (PLP) gene, and is characterized by an almost complete absence of myelin.

The MION-46L-OX-26 complex and cultured CG-4 cells were incubated with the Tfr-targeted as well as unconjugated nanoparticles. The magnetic labeling and resulting uptake of particles was evaluated by (immuno) histochemical staining.

For Prussian Blue staining, cytospins were fixed with 4% glutaraldehyde, washed, incubated for 30 min with 2% potassium ferrocyanide (Perls' reagent) in 6% HCl, washed, and counterstained with nuclear fast red. For immunoperoxidase-staining, cytospins were fixed with acetone, washed, incubated with rabbit-anti-mouse Ig-HRP (DAKO P260, 1:20 diluted, with addition of 3% normal rat serum) for 45 min at room temp, washed, incubated with 0.2 mg/ml solubilized 3-amino-9-ethyl-carbazole in 0.05 M acetate buffer, pH = 4.9 + 0.03% H_2O_2 for 10 min, washed, and counterstained with Mayer's hematoxylin. To determine the relative saturation of OX-26- (MION-46L) uptake, some cytospins were first incubated with 20 µg/ml OX-26 for 60 min at room temp, washed and further processed as described above. No Prussian Blue positive product could be detected in cells incubated

with MION-46L alone, even at a 10-fold higher concentration, indicating a poor opsonization of MION-46L. In contrast, the OX-26-MION-46L tagged cells demonstrated the presence of numerous intracellular vesicles, that were filled with both magnetic nanoparticles and OX-26 moab (Fig. 1).

For transmission electron microscopy (TEM) tagged, washed, and 4% glutaraldehyde-fixed CG-4 cells were embedded in 2% agar, and stained with 0.1% osmium tetroxide for 30 min and with 0.5 % uranyl acetate overnight. Ultrathin sections were further stained with lead citrate, and examined with a JEOL 100CX transmission electron microscope operating at 60 kV for variable-field T1 and T2 relaxometry, tagged, washed CG-4 cells were counted using a hemacytometer, and 1 x 10^7 cells were resuspended in 500 µl 4% w/w gelatin (Sigma G-2500, 300 Bloom) + 0.02 % NaN_3. Using a custom-designed variable field relaxometer (Southwest Research Institute, San Antonio, TX), spanning a Larmor frequency range of 1-64 MHz (corresponding to a field strength of 0.025-1.5 Tesla), the T1 was measured using a saturation recovery pulse sequence. T2 was measured using a Carr-Purcell-Meiboom-Gill pulse sequence with 500 echoes and an interecho time of 2, 4, 6, and 10 msec.

The similarity between the staining pattern for iron particles and the conjugated moab suggests that, at 48h following labeling, both components still remain intact and covalently linked. MION-46L-OX-26 tagged, washed CG-4 cells were also recultured for an additional 5 days. At this time point, cells were still dividing rapidly, and the relative cellular number of Prussian Blue-positive particles was significantly reduced (due to the dilution effect of multiple cell divisions). However, no OX-26 positive vesicles could be observed, indicating an active metabolism process of the antibody, either by (enzymatic) biodigestion inside the vesicle, or, alternatively, by extracellular excretion following Tfr recycling. The normalized (to cellular testing density) T1 and T2 relaxation rates at this time point were, depending on the frequency and interecho time, approximately 10-20 % of the immediate post-tagging values.

The TEM results were typical of receptor-mediated endocytosis, with a reversal of the membrane upon endocytosis of the vesicle. The Tfr-targeted uptake was further analyzed using variable field T1-T2 relaxometry. No significant increase of relaxation rates could be observed for unconjugated MION-46L-incubated cells. Over the entire frequency range, the OX-26-MION-46L incubated cells showed a small but significant increase of 1/T1 (Fig. 2), that was about double the values obtained for unconjugated MION-46L. The difference for

the 1/T2 values of OX-26-MION-46L-incubated cells was much more pronounced, with an increase with frequency that was interecho time (TE) dependent. The TE dependence and enhancement of proton spin dephasing, normally not seen in simple MION-46L solutions (because of their nanometer size), is indicative of (intra) cellular clustering. In this micrometer size regime, either the endocytosed vesicles or the cells themselves act as one, larger particle, that causes additional field gradients for the diffusing water protons. The 1/T2: 1/T1 ratio of about 10 at 1.5 Tesla ratio is therefore several-fold larger than the value seen for non-cell bound MION-46L in solution (13). There was a dose-dependent decrease of the intensity of the Prussian Blue-positive vesicles as well as the magnitude of the 1/T1 and 1/T2 relaxation rates. However, even at the lowest tested dose of 2 μg Fe/ml MION-46L-OX26, the CG-4 cells showed an abundance of iron- and OX-26 containing vesicles, with 1/T2 relaxation rates of in the order of 10 s^{-1} at 1.5 Tesla, compared to control values of about 1 s^{-1}.

Magnetically labeled CG-4 cells were grafted into the spinal cord of neonatal *md* rats and normal littermates. CG-4 cells were incubated with 12 μg Fe/ml MION-46L-OX-26 for 48 h, trypsinized, washed three times, and counted. Using a 30 μm diameter micropipette, approximately 5 x 10^4 cells in 1.0 μl medium were grafted into the T13/L1 region of the spinal cord of 7-day old *md* rats (n=5) and 1 normal littermate. The place of inoculation was marked with charcoal. Grafting experiments with unlabeled cells (n=5) and with magnetically labeled cells that were fixed with paraformaldeyde (n=2) were included as controls. Grafting experiments with unlabeled cells and cells that were labeled but subsequently fixed with paraformaldehyde were included as controls. At either 10 (n=4) or 14 (n=9) days following transplantation, the spinal cord was removed and further processed for 3D high-resolution MR imaging.

Rats were perfused with 4% paraformaldehyde, and spinal cords were dissected and further fixated for several days. Samples were placed in 5 mm NMR tubes, filled with Fomblin LC08 (Ausimont USA, Inc.). 3D multi gradient echo MR images were obtained at 52 or 78 μm isotropic resolution using a 4.7 T Varian INOVA NMR spectrometer and a 6 mm diameter Bruker saddle coil. The scan parameters were: FOV=20x5 mm; matrix 384x96 or 256x64; NEX=100; TR=100 msec; TE = 2.5 or 6 msec; n echoes=6, flip angle = 30 deg. From the raw dataset, both amplitude images, quantitative R2* maps, and differential phase maps were created using IDL processing software. The MR microscopy showed extensive migration (up to 8.4 mm) of grafted cells, particularly in the area of the

dorsal column (Figs 3 and 4). The migration distance for the one normal littermate studied was 10.1 mm (not shown), suggesting that the presence of normal myelin and functional oligodendrocytes does not impede the migration of grafted CG-4 cells in the early developing CNS. In the unlabeled cell (control) graft MR images, no contrast could be observed except a fine hairline representing the 30 μm track of the micropipette. The labeled dead cell control experiments showed contrast only at the injection site (not shown); the area of contrast was less than 0.3 mm, and no spread could be observed. This implies that, even at 14 days post injection, the iron particles remain localized and are not taken up and redistributed by other cells. Calculated differential phase maps proved that the contrast enhancement is caused by dephasing of proton spins in areas of different magnetic field gradients. These areas also correlated directly with calculated 1/T2*.

The MR images were further correlated with histopathological staining for iron, myelin, glial fibrillary acidic protein (GFAP), and microglia. Following MR imaging, the (fixed) spinal cord specimens were cryoprotected and cut at 12 μm slice thickness. Sequential sections were stained for iron using the Prussian Blue reaction, for myelin using anti-PLP antibody, for astrocytes using anti-GFAP antibody, and for microglia using isolectin B4 as described in S.-C. Zhang *et al.*, *J. Neurocytol.* 27, 475 (1998) and S.-C. Zhang et al., *Proc. Natl. Acad. Sci. USA* 96, 4089 (1999). (Figs 3 and 4). Both the Prussian Blue and myelin staining matched closely the area of contrast enhancement seen on the MR images. Since the Prussian Blue reaction and myelin staining did not overlap with GFAP and isolectin B4 staining (not shown), the MION-46L had not been redistributed into astrocytes or microglia cells. The morphology of iron-containing cells resembled that of oligodendrocytes, with a small cell body and multiple processes (Fig 4D). The CG-4 cells appeared to have migrated almost exclusively within the dorsal column, in agreement with the earlier reported results, where a migration distance of up to 7 mm was observed at 2 weeks following transplantation.

These results demonstrate that Tfr-targeted, magnetically labeled oligodendrocyte progenitors fully retain their migrating- and myelinating capacity *in vivo*, and as such do not behave differently from unlabeled cells. This work may have important implications for future studies involving transplantation of glial cells, oligodendrocyte progenitors, and neural stem cells. Since the Tfr is ubiquitous and present on many neural cells in high numbers, the Tfr-targeted magnetic labeling presented here may be easily extended to other neurografting studies. MR tracking of grafted cells will aid in identifying and understanding

the molecular mechanisms which are responsible for the growth and migration of neurotransplanted cells *in vivo*. In particular, this technique may be applied to accurately determine the achieved extent of (re)myelination, and to investigate, on a continuous, non-invasive basis, the various factors which may either promote or inhibit widespread, global myelination.

EXAMPLE 2

Labeling of Thymocytes with Supermagnetic Iron Oxide

Freshly isolated thymocytes and tumor infiltrating lymphocytes from rats can be used as cells. Thymocytes are insolated as a single cell suspension from 4-8-week-old Cr:NIH-mu+ rats by passage of thymus tissue through a fine nylon mesh, followed by centrifugation over a density gradient. For labeling thymocytes are adjusted to 1 x 10^8 cells/ml in phosphate buffered saline and incubated with a biotinylate (glyco) protein followed by incubation by (strept) avidinylated iron oxide, each 15 min. on ice. Cells are then be diluted 1:5 in RPMI-1640 medium without washing, further incubated for 120 min. to allow iron oxide internalization, and washed three times to remove free iron oxide. Cytotoxic T-cells are labeled by a similar method, and can be re-cultured in 24-well plates for up to 7-days.

For evaluation in light microscopy, cell suspensions are stained for iron using Perls Prussian technique. For assessment of internalization, cells may be embedded in agar, stained with osmium tetroxide and uranyl acetate, and examined with transmission electron microscope. Suspensions of cells are prepared in 2% w/w gelatin in PBS for relaxometry at 25^0C from 0.05 to 1.5 T. With a variable-field relaxometer, T1 was measured using a saturation recovery pulse sequence with 32 incremental τ values. T2 is measured using a Carr-Purcell-Meiboom-Gill pulse sequence with 100 decreasing spin-echo intensities and an interecho time of 2 sec.

EXAMPLE 3

Labeling of Blood Cells

Lymphocytes or peripheral blood mononuclear cells (PMNCs), including peripheral blood lymphocytes, tumor-infiltrating lymphocytes, or thymocytes can be used as cells. The cells can also be genetically engineered to produce a compound with anti-tumor activity (e.g. TNF-tumor necrosis factor, interleukins, lymphocyte activation factors, anti-angiogenic factors (e.g. angiostatin, endostatin, VEGF inhibitory analogues). Cells can either be freshly isolated or thawed from frozen state. Cells are incubated for 24-48h in the

presence of dysprosium-dendrimer G=10 (generation 10) conjugated to an internalizing anti-CD antibody. One such antibody is CD 71. Cells are washed 3 times in sterile PBS, centrifuged, resuspended, and injected IV. The vital signs of the host is monitored during infusion to avoid reactions to the cellular infusion. The host is then scanned with MRI in the area the tumor.

EXAMPLE 4

Labeling of Neurons

LBS neurons or neural stem cells are incubated for 24-48 hrs in the presence of magnetoliposomes conjugated to OX-26. The magnetoliposomes may also be encapsulated with NGF. The cells are washed and implanted in humans with substantial motor deficit following a stroke episode. The survival, integration, and motility of these cells is monitored using MRI

EXAMPLE 5

Labeling of Langerhans cells

Langerhans cells are incubated for 24-48 h with a superparamagnetic iron oxide conjugated to the moab 83-14 (anti-human insulin receptor). Following washing, cells are preferably encapsulated into a semipermeable membrane to reduce immuno-rejection. Such membranes are readily available from scientific and pharmaceutical supply companies. An example of such a device is manufactured by Alza, Inc. The membrane does not impair water diffusion through the membrane to the cells and relaxation enhancement. Encapsulated islets (10,000 islets/kg) are then surgically implanted directly into the peritoneal cavity through a midline incision. The cellular settlement and long-term survival up to at least 60 months may be monitored using MRI.

EXAMPLE 6

Labeling of Cell Lines

Carcinoma, sarcoma, or neoplastic cell lines can be labeled by the present invention. Such cells are incubated for 24-48 hrs in the presence of gadolinium-poly-L-lysine conjugated to a growth factor (e.g. EGF, or e.g. autocrine peptides such as bombesin). Cells are washed and injected *in situ*. Growth, motility, and invasiveness are monitored using MRI. This technique provides an assay for compounds that inhibit cell motility and invasiveness. This allows for the non-invasive evaluation of metastasis.

EXAMPLE 7

Labeling Hepatocyte Stem Cells

Hepatocyte stem cells or progenitor cells are incubated for 24-48 hrs in the presence of magnetodendrimer conjugated to transferrin or asialofetuin under conditions described in previous examples. The cells are washed and injected into a cirrhotic liver. Such cells may also be genetically engineered to produce a collagen-dissolving substance. The survival and proliferation of cells leading to the repopulation of liver lobes is monitored using MRI.

EXAMPLE 8

Labeling Viruses

Viruses can also be labeled with MRI sensitive compounds by the method of the present invention. A virus (e.g. HIV-1 virus), with or without the nucleic acids removed, is labeled for 24-48 hrs in the presence of iron-oxides conjugated to an anti-body directed against an internalizing capsid protein. Virus particles are washed and injected IV. The dissemination pattern and integration into the host is tracked with MR.

EXAMPLE 9

3D MR Tracking of Magnetically Labeled Oligosphere Transplants:
In Vivo Labeling in the LE (Shaker) Rat Brain

Introduction

Multipotent stem cells are present in the nervous system *(R. McKay, Science 276, 66 (1997))*, and have recently been isolated from the human brain *(P.S. Eriksson, et al., Nature Med. 4, 1313 (1998))*. They can be established as permanent cell lines and induced to become oligodendroglia cells in the form of free-floating oligospheres following growth factor treatment *(S.-C. Zhang et al. J. Neurocytol. 27, 475 (1998))*. The adult brain retains the potential to generate these type of cells, and following transplantation they have proven to exhibit extensive myelination capacity *(S.-C. Zhang, et al., Proc. Natl. Acad. Sci USA 96, 4089 (1999))*. It has also been demonstrated that by incorporating a magnetic label into glial cells it is possible to visualize cell spread and the induced myelination non-invasively using MRI *(J.W.M. Bulte et al., Proc. Natl. Acad. Sci. USA, 96(26): 15256-61, (1999))*. Using magnetically labeled oligospheres, these cells can be monitored *in vivo,* repeatedly. This allows researchers to make a "snapshot" of their precise spatial migration and distribution at a particular given time. This development is of crucial importance if such stem cell-based therapies are going to be pursued in humans.

Materials and Methods

Oligospheres (OS) were prepared as described *(S.-C. Zhang et al., J. Neurocytol. 27, 475 (1998))*, and transfected with the LacZ reporter gene in order to follow cell migration histochemically following MRI. OS were co-cultured with MION-46L-OX-26 *(J.W.M. Bulte et al., Proc. Natl. Acad. Sci. USA, 96(26): 15256-61, (1999))* or a rhodamin-fluorescent derivative at a dose of 25 μg Fe/ml for 24h. In this way, magnetic tagging is achieved by specific targeting and uptake through the transferrin receptor. Magnetic tagging was always verified by Prussian Blue staining. Approximately 1-1.5 x 10^5 labeled OS cells in 2-3 μl medium were transplanted into the intracerebroventricular region *(B. Yandava et al., Proc. Natl. Acad. Sci. USA 96, 7029 (1999))* of neonatal (P0) Long Evans (LE) shaker rats and normal littermates (n=17).

Rat pups were imaged at 1.5 T using a quadrature wrist coil with several different T1w, PD/T2W, FSE, and T2*w pulse sequences. 2D images were obtained at 2 mm slice thickness, and a 1.2 mm thick 3D SPGR data set was obtained. In order to allow repeated scanning, all scans were performed using isoflurane anesthesia. 3D multi gradient echo MR images were also obtained at 313 μm isotropic resolution using a 4.7 T GE CSI Omega NMR spectrometer and a 1.5 inch diameter home-made surface coil. The 4.7 T scan parameters were: FOV=6x3x3 cm; matrix=192x96x96; NEX=1; TR=100 msec; TE= 6 msec; n echoes=6, flip angle = 15 deg.

Results and Discussion

The first images at approximately 2 weeks post-transplantation showed a localized distribution of cells around the injection site. The injection site could always be identified by its "blooming effect" on gradient echo images. At 3-4 weeks post-transplantation, further cellular spread could be observed inside the white matter areas of the corpus callosum, internal capsule, and hippocampus (Fig. 6). The extent of cellular spread in normal littermates was greater than for the shaker animals; a similar finding was observed previously for the spinal *(J.W.M. Bulte et al., Proc. Natl. Acad. Sci. USA, 96(26): 15256-61, (1999))* Since shaker animals are myelin-deficient and oligodendrocytes stop dividing once they myelinate, a non-limiting explanation is that the reduced temporal-spatial migration in the shakers can be explained by a cellular "switch-off" that interferes with cellular expansion and motility. Studies of the long-term survival and migration kinetics are currently in progress.

Conclusions

These results are a first demonstration of the feasibility of dynamic MR imaging (dMRI) of migrating cells relevant to therapeutic intervention. Moreover, this approach has proven to also work using a clinically approved magnet and field strength, and may thus help guide further advances in stem-cell based therapies.

EXAMPLE 10

Cellular Imaging: Assessment of Intra-Neoplastic Growth Pattern Using Magnetic Resonance Labeled Tumor Cells

This experiment was designed to demonstrate that by implanting magnetically labeled tumor cells it should be possible to monitor the intratumoral cellular growth pattern repeatedly, in order to take a ultrastructural "snapshot" of neoplastic development.

Method/Materials

Human small cell lung carcinoma (SCLC) cells were labeled magnetodendrimers, a new cellular MR contrast agent, at 10 ug Fe/ml for 48h. Approx 5 E6 tagged cells were implanted sc in the flank of nude rats (n=6), along with untagged cells as control (n=6). Tumor growth was followed in vivo repeatedly on a weekly basis using a clinical 1.5 T system, and ex vivo using a 4.7 T magnet at 50 micron isotropic resolution. Histopathologic correlations were done using (DAB-enhanced) Prussian Blue stains.

Results

Both groups of implanted cells: labeled or "tagged" and un-labeled or "untagged" cells exhibited similar growth rates, indicating that the contrast agent can be considered a "vital dye". At the earlier timepoints (tumor size approx. 5-10 mm) the neoplams appeared uniformly black on the images. At later timepoints (tumor size approx. 10-30 mm) bizarre migration patterns were seen, mainly from the dark center protruding towards the outer rim, with distinct clusters of hypointense labeled cells. The outer rim of the tumor usually lacked hypointense cells and was isointense to the untagged controls, as a result from multiple cell divisions and associated MagTag-dilution effects. The high-resolution *ex vivo* images demonstrated organized contrast patterns with multiple single pixel spreads. (DAB-enhanced) Prussian Blue staining matched the areas of MR contrast and showed that each pixel corresponded to a single cell, as a result from outer sphere susceptibility-enhancement effects.

Conclusions

These results demonstrate the feasibility of (single) cell imaging as it relates to intratumoral growth patterns. This technique may have profound implications for the assessment of therapeutic strategies directed at eradicating "dormant" tumor cells, e.g. anti-angiogenic therapy. For instance, the majority of cells appear to become dormant at about 3 weeks after implantation. Then, after a certain period of time, they became activated and began to move along specific patterns to the periphery of the tumor. The method of the present invention allows scientists to uniquely monitor this the phenomenon. By comparison, previous investigators have labeled tumor cells with transfected GFP (green fluorescent protein) or lacZ. In their studies all the tumor cells express the label the same (since it is homogeneous protein expression). The label of the present invention becomes diluted and, from the dilution effect the growth pattern is can be observed. Figs. 7 and 8 illustrate this phenomenon.

An alternative method of labeling cancer cells is to inject the compound, I.V., into a feeding vessel of the tumor. This technique labels cells the by means of non-specifically "leaky" vessels in the tumor. For these techniques the amount of magnetodendrimers to be injected will depend on the volume of the area to be labeled and the concentration Fe label in the composition. A skilled practitioner will calculate the amount from the required density of label.

EXAMPLE 11

Labeling Neural Stem Cells

Magnetodendrimers can be used to label neural stem cells, *in situ*, as a method of studying them and tracking their behavior. Magneto dendrimers are made acceding to standard procedures and solubilized in sterile saline. Monkeys are the experimental model. Magneto dendrimer solution is infused into the sub ventricular ependyma by stereotaxic surgery using a Hamilton syringe or by exposing the surface of the cortex and positioning the syringe under visual guidance. The location of the dendrimers in the ependymal layer is confirmed by MRI immediately after surgery.

Monkeys are then observed with MRI on a regular schedule to observe possible activation and migration of neural stem and progeny cells. Proliferation or migration will be correlated to other events, such as aging or injury. Verification that dendromer labeled cells are neural is made histologically, post mortem. Brain tissue is sectioned, mounted, and stained with a neural specific stain, for example nestin. Sections may be counterstained

to help localize the labeled cells. Activation of neural stem cells could be an important therapeutic strategy for neuro-degenerative disease and neural injury. Observing cells allows evaluation of methods of activating cells, which could be used to treat disease.

EXAMPLE 12

Labeling Rat Breast and Lung Carcinoma Cells.

Rat breast and lung carcinoma cells were tracked so as to label with magnitodendrimers *ex vivo* for 48 h with 10 μg Fe/ml magneto dendrimers. Labeled cells were injected iv and animals were followed with MRI on a daily basis. Since single magnitodendrimer labeled cells can now be detected in tissue (e.g. JWM Bulte *et al.*, RSNA 2000), very early metastases can be detected. Thus, the very first marginating and nestling cells can be detected.

EXAMPLE 13

Labeling Glial Cells

The brain and spinal cords from E45 male canine fetuses were collected following cesarean section. A mixed glial cell preparation was separated using standard culture methods. Briefly brains and spinal cords are rapidly removed under sterile conditions, chilled, minced with surgical scissors, and cultured for four weeks to allow neurons to die off. Glia were subcultured and was labeled with magnetodendrimers at 10g Fe/ml for 48h. The labeled cells were then transplanted in 9-13-day old *sh* pups. Animals were monitored using MRI and the observed contrast matched the histopathological stainings for new myelination.

EXAMPLE 14

Labeling Embryonic Tissue

Mouse ES cell embryoid bodies were labeled with magnetodendrimers by incubation for 24 hr. Embryoid bodies were then transplanted into adult mice, 9 days after traumatic spinal cord injury (contusion). The observed MRI contrast 2-5 weeks later corresponded to the histological analysis and demonstrated that labeled ES cells survived and differentiated into astrocytes, oligodendrocytes and neurons, and migrated as far as 8 mm away from the lesion edge. This coincided with overall improvement in limb coordination, gait and stability.

EXAMPLE 15

Hematopoietic Stem Cells

Hematopoietic stem cells (CD 34+) were labeled with magnetodendrimers by incubating cells with dendrimers for 24 hours. CD34+ cells were separated from bone marrow by standard methods. Cells are then injected i.v. into host animals. Cells could be detected in the reticulo-endothelial system (bone marrow, liver and spleen).

EXAMPLE 16

Biotinylation, crosslinking, and internalization of cell surface proteins using streptavidinylated MR contrast agents.

Internalization of drugs/ligands including magnetic nanoparticles is often a result of receptor crosslinking, with the receptor sending a complex (through G proteins) internalizing signal to the cell. The multiple (4) binding sites of streptavidin for biotin allow crosslinking not only of soluble biotinylated molecules but also of biotinylated receptor molecules that are present on the cell surface (*U Wojda et al., Bioconj. Chem., 10, 1044-1050, 1999*). Cells were incubated in a final concentration of 0.5 ng of sulfo-NHS-biotin/cell in PBS for 30 min at 4°C and washed twice with ice cold PBS. Avidin-coated or streptavidin-coated magnetic nanoparticles were then incubated with the cells (1E6 cells/ml PBS) for 30 min at 4C and the cells were transferred to 37°C and incubated for 2h. Prussian Blue staining and relaxometry revealed a very high degree of labeling.

All references cited herein are specifically hereby incorporated by reference. Having described the invention in detail and by reference to the preferred embodiments it will be apparent to those skilled in the art that modifications and variations are possible without departing from the scope of the invention as defined in the following appended claims.

CLAIMS

1. A method of labeling living cells to render such cells magnetic resonance sensitive comprising:
   a. treating a magnetic resonance sensitive agent to render it capable of being internalized by the cells;
   b. bringing the treated, magnetic resonance sensitive agent into contact with the cells to be labeled;
   c. allowing the magnetic resonance agent to be internalized by the cell; and
   d. rendering cells magnetic resonance sensitive.
2. The method of labeling living cells of claim 1, wherein the magnetic resonance sensitive agent is a superparamagnetic agent.
3. The method of labeling living cells of claim 1, wherein the magnetic sensitive agent is selected from the group consisting of gadolinium chelates and iron oxide.
4. The method of labeling living cells of claim 1, wherein the magnetic sensitive resonance agent is selected from the group consisting of (magneto) ferritins, (magneto) liposomes, (magneto) dendrimers, dysprosium, gadolinium-containing macromolecular chelates, and iron-containing macromolecular chelates.
5. The method of labeling living cells of claim 1, wherein the magnetic sensitive resonance agent is dysprosium-dendrimer.
6. The method of labeling living cells of claim 2, wherein the superparamagnetic agent is MION-46L.
7. The method of labeling living cells of claim 2, wherein the superparamagnetic agent is a magnetodendrimer.
8. The method of labeling living cells of claim 1, wherein the method of treatment of the magnetic sensitive agent comprises conjugating it to a targeting compound.
9. The method of labeling living cells of claim 8, wherein the targeting compound is an antibody.
10. The method of labeling living cells of claim 9, wherein the antibody is selected from the group consisting of: 83-19, JSB-1, MRK-16, C219, OX-26, B3/25, T56/14, OKT-9, L5.1, 5E-9, R17-217, T58/30, 8D3, and R17-217.
11. The method of labeling living cells of claim 9, wherein the antibody is OX-26
12. The method of labeling living cells of claim 9, wherein the antibody is 83-14.

13. The method of labeling living cells of claim 9, wherein the antibody is an anti-CD antibody.

14. The method of labeling living cells of claim 1, wherein the cells are selected from the group consisting of stem cells , neural stem cells, bone marrow cells, hematopoietic cells, tumor cells, lymphocytes, leukocytes, granulocytes, hepatocytes, monocytes, macrophages, fibroblasts, mesenchymal cells, bacteria, and neural cells.

15. The method of labeling living cells of claim 1, wherein the cells are stem cells.

16. The method of labeling living cells of claim 1, wherein the cells are neural cells.

17. The method of labeling living cells of claim 1, wherein the cells are thymocytes.

18. The method of labeling living cells of claim 1, wherein the cells are lymphocytes.

19. The method of labeling living cells of claim 1, wherein the cells are Langerhan's cells.

20. The method of labeling living cells of claim 1, wherein the cells are hepatocytes.

21. The method of labeling living cells of claim 1, wherein the cells are viruses.

22. The method of labeling living cells of claim 1, wherein the cells are selected from the group consisting of: carcinoma, sarcoma, and neoplastic cells.

23. The method of labeling living cells of claim 8, further comprising the step of allowing the targeting compound to bind to a receptor on the surface of the cell, wherein the receptor is selected from the group consisting of: hormone, cytokine, and growth factor receptors.

24. The method of labeling living cells of claim 23, wherein the receptor is selected from the group consisting of the group of insulin receptors, p-glycoprotein receptors, Na^+ /K^+ ATPase receptors, ferritin receptors, lactoferrin receptors, transferrin receptors, VEGF, BDNF, IGF, IL-2, EGF, NGF, and PDGF receptors.

25. The method of labeling living cells of claim 23, wherein the receptor is selected from the group consisting of insulin receptor, and transferrin receptor.

26. A method of detecting pathology in a host suspected of having pathology caused by malfunctioning of cells comprising:

a. labeling living cells with a magnetic resonance sensitive agent;

b. introducing such cells into the host; and

c. imaging the host with MRI so as to locate the cells within the host.

27. The method of detecting pathology of claim 26, wherein the cells are carcinoma cells.

28. The method of detecting pathology of claim 26, wherein the host is an experimental animal.

29. A diagnostic kit for labeling living cells which are to be introduced into a host for experimental, diagnostic, or therapeutic purposes, comprising:

a. a magnetic sensitive agent which has been treated to render it able to be internalized by the cells;

b. sterile solutions comprising one or more of: buffers, salts, proteins, nutrients, indicator dyes, and preservatives; and

c. sterile containers to bring the magnetic sensitive agent into contact with the cells.

30. A method of detecting living cells in a host by labeling them with magnetic sensitive agents comprising:

a. treating the magnetic resonance sensitive agent so as to render it capable of being internalized by the cells;

b. bringing the magnetic resonance sensitive agent into contact with the cells;

c. allowing the magnetic resonance agent to be internalized by the cells;

d. rendering the cells magnetic sensitive, and

e. imaging the host with MRI so as to detect the labeled cells.

31 The method of labeling living cells of claim 30, further comprising the steps of labeling cells in vitro and then applying the cells to the host.

32 The method of labeling living cells of claim 30, further comprising the step of introducing the treated magnetic resonance sensitive agent into the host so as to label cells located at a site within the host.

33 A method of labeling living cells to render them magnetic resonance sensitive comprising:

a. coating magnetic nanoparticles with one of strepavidin or avidin;

b. biotinylating the cells;

c. contacting the cells with the coated nanoparticles;

d. allowing the cells to internalize the nano-particles; and

e. imaging the cells with MRI.

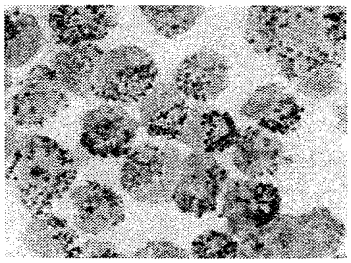

FIG. 1D

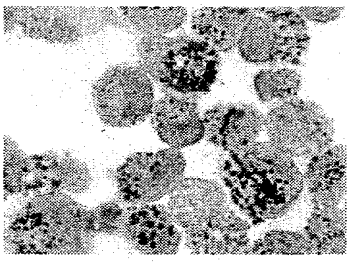

FIG. 1C

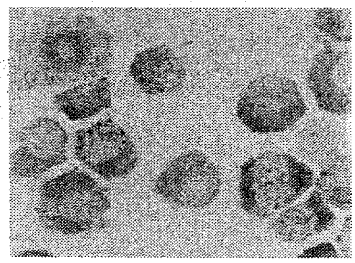

FIG. 1B

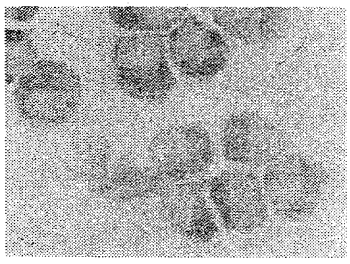

FIG. 1A

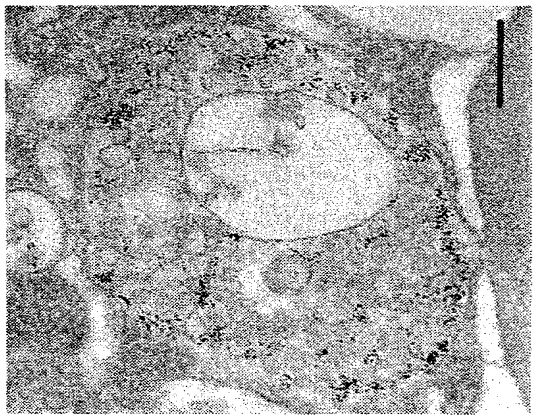

FIG. 1F

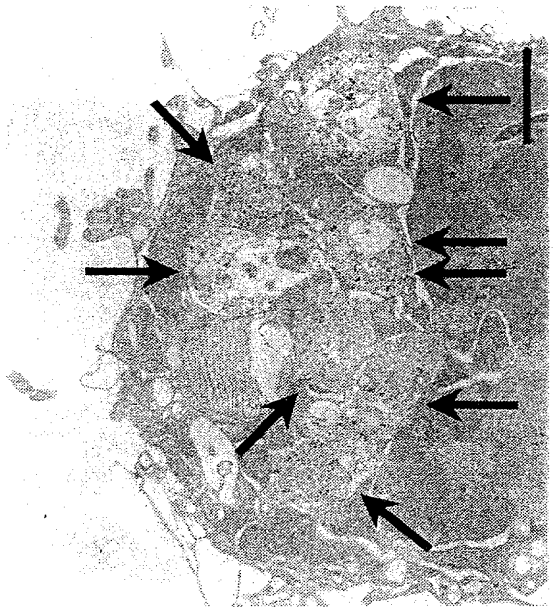

FIG. 1E

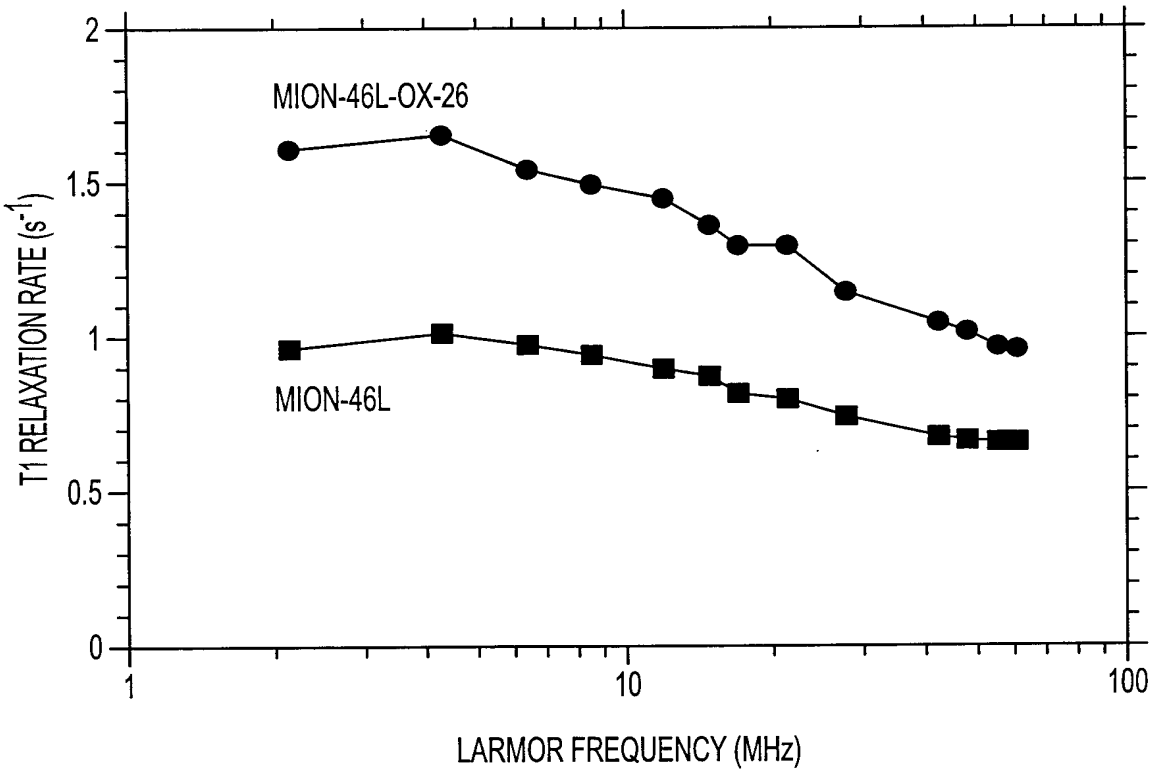
MION-46L-OX-26
MION-46L
T1 RELAXATION RATE (s^{-1})
LARMOR FREQUENCY (MHz)
0
0.5
1
1.5
2
1
10
100

FIG. 2A

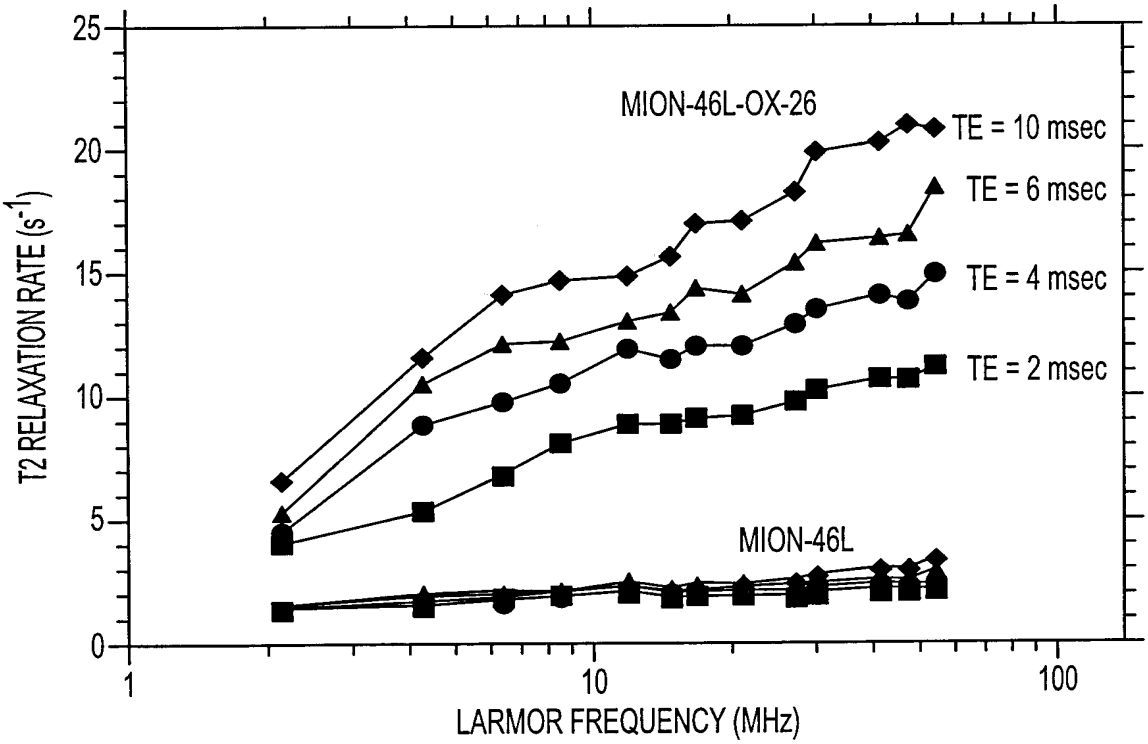
MION-46L-OX-26
TE = 10 msec
TE = 6 msec
TE = 4 msec
TE = 2 msec
MION-46L
T2 RELAXATION RATE (s^{-1})
LARMOR FREQUENCY (MHz)
0
5
10
15
20
25
1
10
100

FIG. 2B

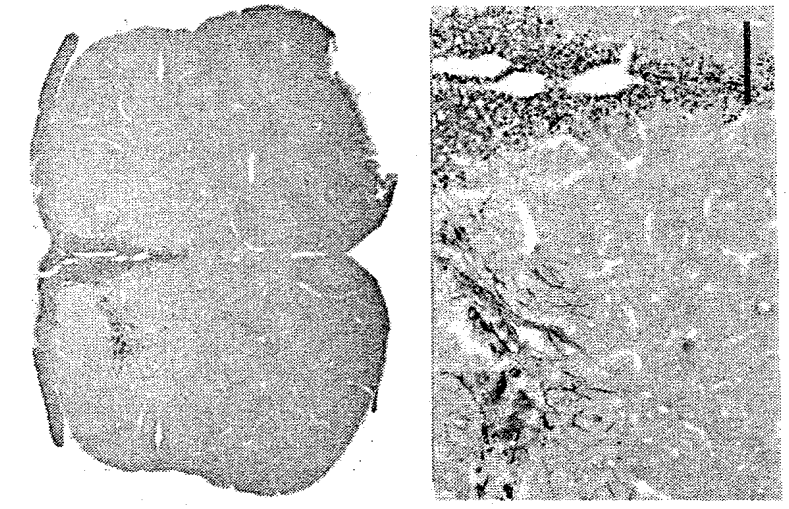

FIG. 3C

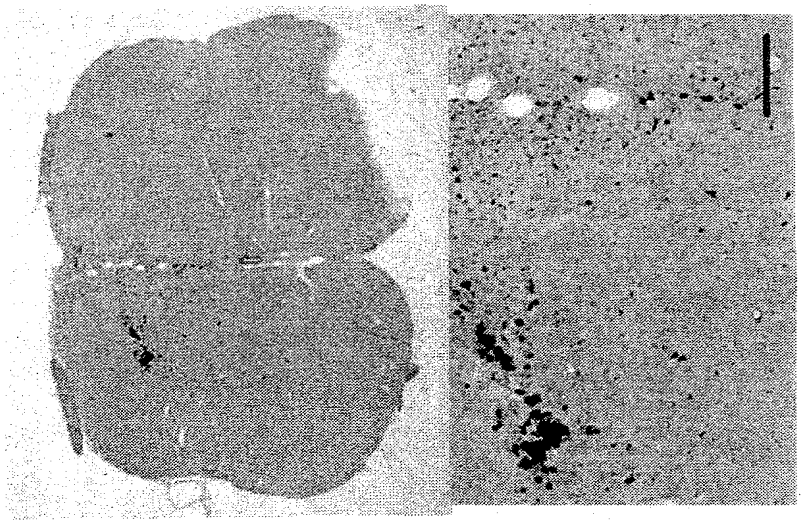

FIG. 3B

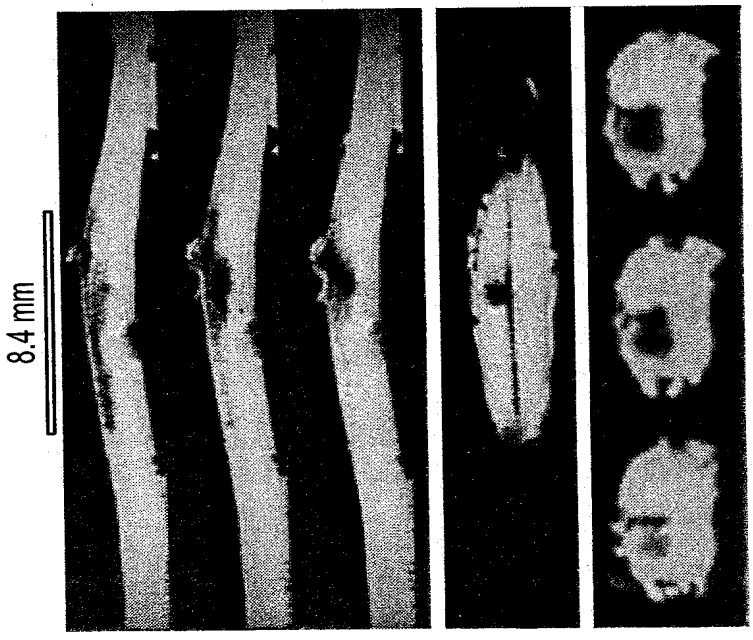

FIG. 3A

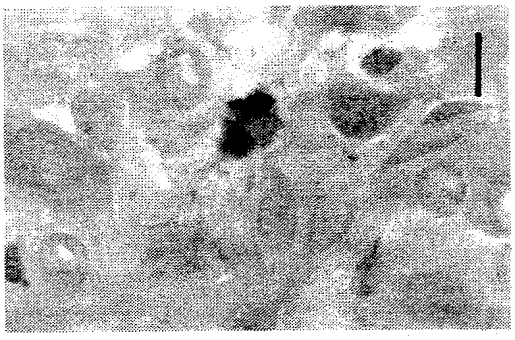

FIG. 4D

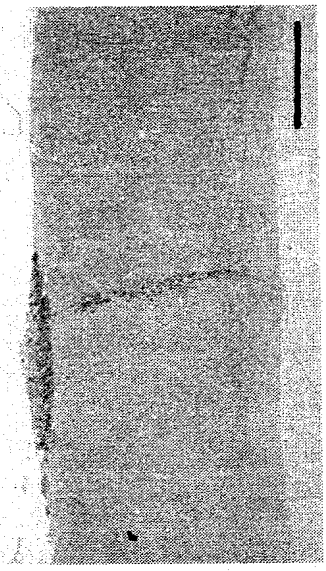

FIG. 4B

FIG. 4C

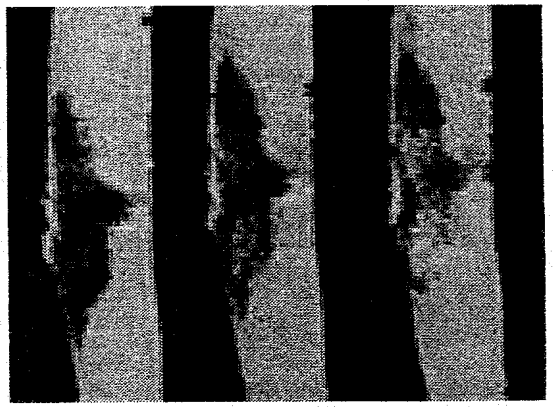

FIG. 4A

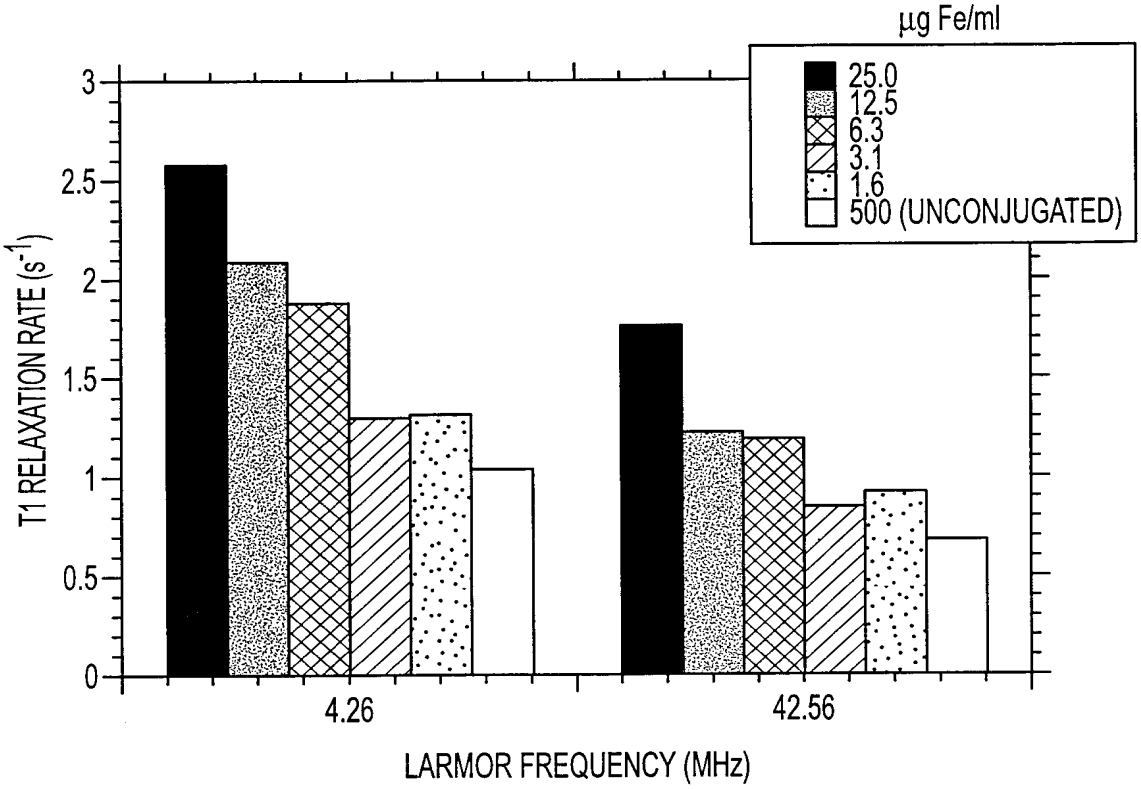
µg Fe/ml
25.0
12.5
6.3
3.1
1.6
500 (UNCONJUGATED)
T1 RELAXATION RATE (s^{-1})
0
0.5
1
1.5
2
2.5
3
4.26
42.56
LARMOR FREQUENCY (MHz)

FIG. 5A

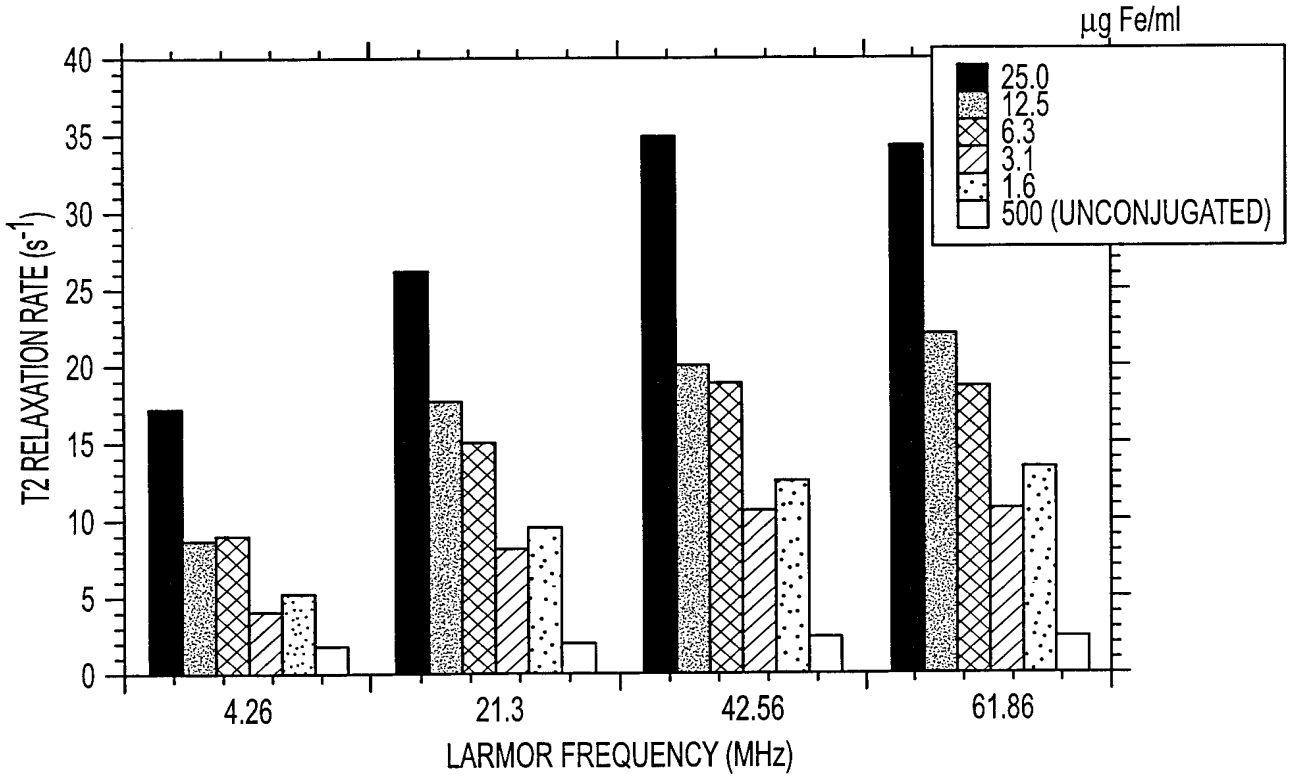
µg Fe/ml
25.0
12.5
6.3
3.1
1.6
500 (UNCONJUGATED)
T2 RELAXATION RATE (s^{-1})
0
5
10
15
20
25
30
35
40
4.26
21.3
42.56
61.86
LARMOR FREQUENCY (MHz)

FIG. 5B

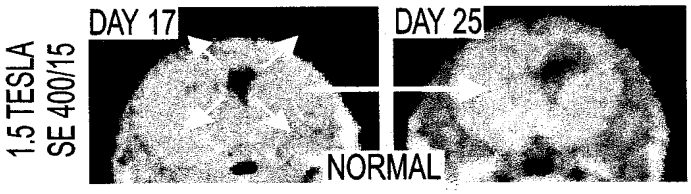

FIG. 6A

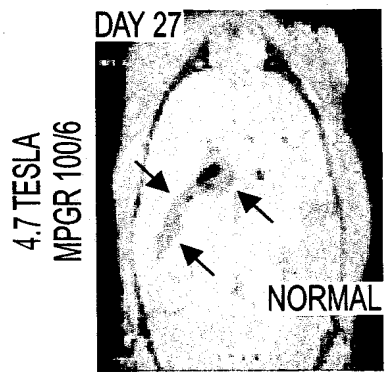

FIG. 6B

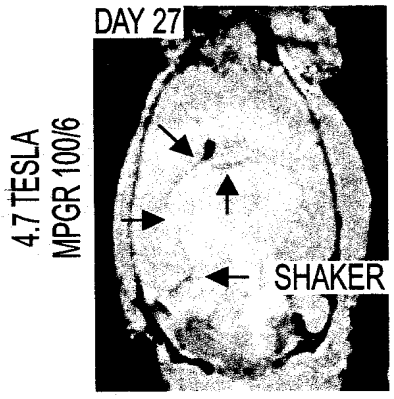

FIG. 6C

27 DAYS FOLLOWING IMPLANTATION

FIG. 7A

20 DAYS FOLLOWING IMPLANTATION

FIG. 7B

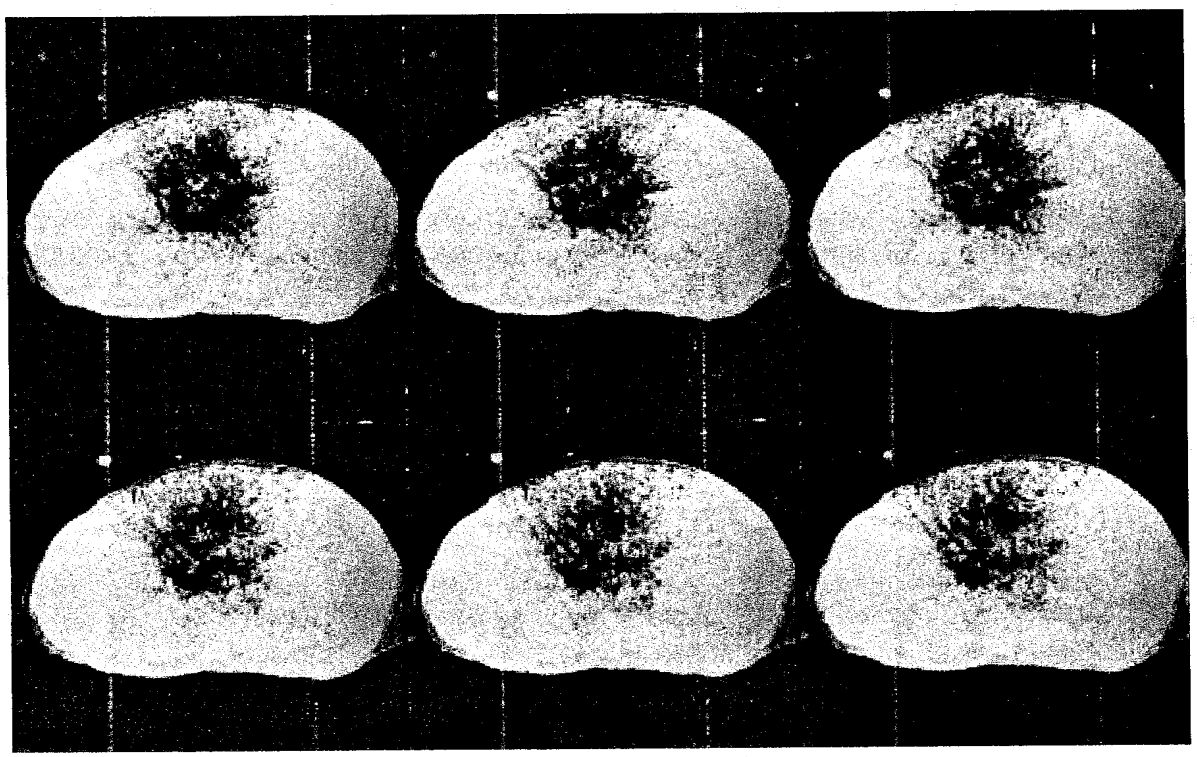

FIG. 8